Official Regulatory Protocol for Nurseries Containing Plants Infected with *Phytophthora ramorum* (Sudden Oak Death)
Revised 15 October 2004

Intended Use:
In February 2002, USDA Animal and Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ) issued a federal domestic regulation for interstate movement of *Phytophthora ramorum* (7 CFR 301.92). The complete text and other information may be found at the USDA APHIS PPQ web site: www.aphis.usda.gov/ppq/ispm/sod/

Since the regulations were first published, *P. ramorum* has been detected in several nurseries outside the quarantined area. These detections catalyzed the need for a standard protocol for use by state and federal regulators to respond to new finds of *P. ramorum* in nurseries outside of the quarantine area. To ensure that there is consistency in responding to new infections of *P. ramorum*, this protocol describes the official activities performed within and surrounding nurseries by USDA APHIS staff in cooperation with state agriculture regulatory officials.

The goal of this protocol is to ensure that any infestations of this serious pathogen are consistently and effectively addressed, mitigated, and eradicated. Cooperation by nursery management personnel is essential. Early detection and reporting of potential *P. ramorum* infections are critical to ensure that spread is contained. The strategies employed in this protocol are similar to those of the European Union and of other areas where eradications are being carried out with measures that ensure rapid suppression of infection, and which prevent the spread of the pathogen.

P. ramorum infestations in nurseries may be introduced via three critical pathways.
- The movement of infected plant material from one nursery to another;
- The natural environmental movement of spores from a nursery or infected wild plants to infect plants in a nursery;
- The transmission of the disease from non-plant pathways to plant material (e.g. the introduction of infested soil, water, potting media, equipment, etc.)

Definitions:

Associated plants: Plants listed in the "APHIS List of Plants and Associated Plants" as "associated plants". These have been reported associated with *P. ramorum* and have not had Koch's Postulates completed, reviewed and accepted by APHIS. These plants are also referred to in the regulations as "associated articles."

Buffer Zone: Area identified as a 10 meter radius from the destruction block (see Appendix 10).
(Use of this term is an adaptation from a FAO definition: An area in which a specific pest does not occur, or occurs at a low level and is officially controlled, that either encloses or is adjacent to an infested area, an infested place of production, a pest-free area, a pest-free place of production or a pest-free production site, and in which phytosanitary measures are taken to prevent spread of the pest [ISPM Pub. No. 10, 1999])

Destruction block: Block of plants to be destroyed. Within a nursery, this is a contiguous block of host plants and/or associated plants containing one or more plants known to be infected with *P. ramorum*. The block will be considered contiguous until there is a 2 meter break of either no plants or no hosts or associated plants.

Federal Order: A signed Order of an emergency nature issued to address a pest situation requiring immediate attention. These are intended to be quickly replaced by an interim rule. To date, Orders have been issued in April and September of 2004. These are posted and may be viewed at: http://www.aphis.usda.gov/ppq/ispm/sod

Free from: Of a consignment, field or place of production, without pests (or a specific pest) in numbers or quantities that can be detected by the application of phytosanitary procedures. (FAO, 1990), FAO-CEPM, 1994.

Host plants: Plants listed in the "APHIS List of Plants and Associated Plants" which have been found associated with *P. ramorum* and have had Koch's Postulates completed, reviewed and accepted by APHIS.

Infected plants: Plants with or without soil verified as being infected with *P. ramorum* based on the use of APHIS approved diagnostics.

Nursery/Facility: Any location where nursery stock is grown, propagated, stored, or sold; or any location from which nursery stock is distributed to a customer.

Nursery site quarantine: For nurseries with host plants or associated plants in the buffer zone. This is a period of time during which host plants and associated plants are prohibited movement within or out of the "buffer zone" (see Appendix 10). This quarantine period begins when the Nursery Delimitation Survey is completed and lasts until such time as both plant parts and environmental conditions conducive to disease expression have occurred for at least 90 days, and inspection, sampling, and testing reveals no further detection of *P. ramorum* within the hold block. Conducive environmental conditions exist when climate conditions match optimum disease etiology and are likely to express disease symptoms 50% or more of the time (see Addendum III in the National Nursery Survey Manual for monthly climatic maps).

Occurrence: The presence in an area of a pest, officially reported to be indigenous or introduced, and not officially reported to have been eradicated. (FAO, 1990), FAO-CEPM, 1994. (compare: Outbreak)

Outbreak: An isolated pest population recently detected and expected to survive for the immediate future. FAO-CEPM, 1994. (compare: Occurrence)

Parallel Quarantine: A quarantine imposed by a State or local plant regulatory authority that is substantially the same as a federally promulgated quarantine.

Quarantine Area: Any State, or any portion of a State, listed in § 301.92-3(c) or otherwise designated as a quarantined (infested) area in accordance with § 301.92-3(b). Articles may be restricted (prohibited) or regulated (conditionally certifiable) from a quarantine area.

Regulated Area: Any State, or portion of a state, in which only nurseries that ship hosts or associated plants interstate are affected and the only affected article is nursery stock. These areas are detailed in the Federal Order posted at http://www.aphis.usda.gov/ppq/ispm/sod

Suspected infected plants: These are plants with visible symptoms of *P. ramorum* infection; and/or host and associated plants that are a part of an infested block or derived from an infested block or buffer zone; and/or plants that have tested postitive using PCR or culturing, but have not been confirmed positive for *P. ramorum* by APHIS.

Trigger Events for Use of Protocol

This protocol should be implemented by APHIS-PPQ and/or its State Plant Regulatory cooperators when the presence of *P. ramorum* has been confirmed in a U.S. nursery from samples collected as part of a trace forward survey*, National *P. ramorum* survey*, or found by other means. Confirmed samples must have been analyzed using a methodology approved by APHIS at an APHIS laboratory.

*See www.aphis.usda.gov/ppq/ispm/sod for links with details on trace forward surveys and the National *P. ramorum* survey.

Authorities

- For States with parallel quarantines for *P. ramorum*, specific actions required by this protocol within and around the nursery are expected to be conducted by the State personnel with Federal support.
- For States without parallel quarantines for *P. ramorum*, specific actions required by this protocol within and around the nursery will be conducted under Federal authority, in cooperation with State personnel.

Communication/Notification

- Communicate suspect finds as soon as one of the following has occurred:
    1. a positive PCR find
    2. a culture that matches the morphology for *P. ramorum* (i.e. isolation of *P. ramorum*)
    3. A discussion with a nursery that positive stock may have been shipped to a neighboring state.

- Immediately provide notification to the owner. Notification details are provided under "Secure the Nursery" section.

- Immediately notify the State Plant Health Director (SPHD) and the State Regulatory Official (SPRO) of the State in which the nursery is located.

- APHIS, PPQ Regional Office and National Headquarters Office shall be notified. See Appendix 7, Resource and Contact List.

- Notify state plant regulatory officials (SPHD's and SPRO's). If possible, SPROs should notify facilities that are impacted by the trace backs and trace forwards. See Survey and Investigate Section.

- **Notification of laboratory results:** Laboratories need to notify the submitter, the SPHD, and the SPRO, the Regional Office and National Program Manager. Ideally the SPRO should notify the owner of the nursery, but either the SPRO (if State authority is used) or the SPHD (if Federal authority is used) may notify the owner of the nursery.

- **Public Notification:** The SPRO and SPHD will use state channels, including public affairs offices to make any public announcements, as necessary. The SPHD will insure that the USDA APHIS Office of Legislative and Public Affairs is aware of the pending release, via the Regional Office and National Headquarters Office.

Secure the Nursery

- If plants not on hold are showing symptoms indicative of *P. ramorum,* they may not be removed from the nursery, from any holding area in the nursery, or moved within the nursery until they are found free of *P. ramorum* or the nursery is officially declared free-from *P. ramorum* and removed from quarantine status or unless approved by a State or Federal Regulatory Official. Any plants confirmed by APHIS to be infected with *P. ramorum* will be destroyed as per this protocol:
- When samples collected from suspected infected plants during trace forward survey, national SOD survey, or other surveys are first confirmed to be infected with *P. ramorum* using diagnostic protocols approved by USDA APHIS PPQ*:*
    - In the case of nurseries that **DO SHIP** interstate, all genera of host plants and associated host plants must be held until delimitation within the nursery is complete. (Note: The hold on genera makes this protocol consistent with the PPQ's Federal Order). This hold may also include "any other product or article that an inspector determines to present a risk of spreading *Phytophthora ramorum,* if an inspector notifies the person in possession of the product or article that it is subject to the restrictions in the regulations" (7CFR part 301.92-2) within the infested facility.
    - In the case of nurseries that **DO NOT SHIP** interstate, only host plants and associated plants need be placed on hold until delimitation within the nursery is complete. This hold will also include growth media and "any other product or article that an inspector determines to present a risk of spreading *Phytophthora ramorum,* if an inspector notifies the person in possession of the product or article that it is subject to the restrictions in the regulations" (7CFR part 301.92-2) within the infested facility.
    - PPQ form 523, Emergency Action Notification will be used as the official Federal authorization of hold. The required treatments and/or basic sanitary and precautionary measures (e.g. bio-containment of suspected infected material, etc.) should be included in the PPQ form 523.

- If the State initiated action, then the appropriate State notification would be used. Stop Sales notices should be placed on the nursery by the appropriate State Regulatory Official.

- Following completion of a Nursery Delimitation Survey:
  - For nurseries with host plants or associated plants in the "buffer zone" (see Appendix 10), these host plants and associated plants are prohibited movement within or out of the buffer zone during the quarantine period. This quarantine period begins when the Nursery Delimitation Survey is completed and lasts until such time as both plant parts and environmental conditions conducive to disease expression have occurred for at least 90 days, and inspection, sampling, and testing reveals no further detection of *P. ramorum* within the hold block. Conducive environmental conditions exist when climate conditions match optimum disease etiology and are likely to express disease symptoms 50% or more of the time (see Addendum III in the National Nursery Survey Manual (can be found at: http://www.aphis.usda.gov/ppq/ispm/sod) for monthly climatic maps).

  - During the quarantine period within the 10 meter buffer zone:
    - Growers will discontinue applications of fungicides for *Phytophthora* control.
    - Plants will be visually inspected a minimum of two times, once about half-way through the anticipated quarantine period and once near enough to the end to have test results coincide with the end of the quarantine period.
    - Plant samples will be collected according to the protocol detailed in Appendix 4 (Plant Sampling Protocol).
    - If water, soil, and/or media samples tested positive for *P. ramorum* during the delimiting survey, samples of the infested water, soil, and/or media material will be resampled and tested during each of the two quarantine period plant inspections.
    - If a plant sample tests positive for *P. ramorum*, both the 10 meter buffer zone and quarantine period must be re-determined, that is the nursery must be re-delimited and the quarantine period must be reset.
    - If water is found to be positive, then any portion of the nursery that has been irrigated with *P. ramorum* infested water is placed on hold.
    - If a soil sample or media sample is found to be positive, then any plants in the block with the infested soil is placed on hold.

Survey and Investigate

- **Delimiting Survey**. Inspect all decorative (established plants within the nursery) and plants (held, for sale or under propagation) of host and associated host genera in the nursery. Plant samples will be collected according to the protocol detailed in Appendix 4 (Plant Sampling Protocol).
- Samples must be analyzed using a methodology approved by APHIS (see Appendix 3). Samples for official confirmation should be submitted to United States Department of Agriculture, National Plant Germplasm Laboratory (NPGPL) in Beltsville, MD (see Appendix 7, Dr. Mary Palm).
- **Trace Forward Investigation.** Initiate trace forward investigations. Identify shipments made prior to the discovery of *P. ramorum*. Notify your PPQ Regional Office of all

shipments made within the 12 months prior to the first positive detection of *P. ramorum* at the nursery. This includes ALL hosts and associated plants in the nursery, not just those found infected. Suspect infected plants identified through trace forwards, that have been moved into a landscape environ, should be inspected and tested during optimal conditions for growth and development of *P. ramorum* symptoms.

- **Trace Back Investigation.** Determine the origin of all infected plant**s** through trace backs. Trace back the plants to point of origin (propagator). The goal is to determine the site of infection. Wholesale nurseries and other places the plants were located should be inspected going back all the way to the propagation site. **Forward this information to your PPQ Regional Office.** After traces are completed, examine all plants received from trace back sources.
- **Soil and Potting Media Sampling.** Determine if infected plant material may have contaminated soil or potting media used at the nursery. Soil from within the destruction block, the 10 meter buffer zone and any downhill blocks must be sampled. Determine the content, origin (constituency), storage and handling of soil or potting media used in the facility. See Appendix 5 for detailed soil and potting media sampling protocol. Keep soil samples separate from potting media samples.
- **Water Sampling.** Determine the source of water used at the facility. Note source of irrigation water and where drainage water flows. Note the type of irrigation system(s) in use, areas of standing water and any safeguards against water back flow in the irrigation system, and any water treatment practices. See Appendix 6 for detailed water media sampling protocol.
- **Cull piles**. Record the location of any cull piles that may be contaminated with infected plant material or associated soil and/or potting media. Check any cull piles for *P. ramorum* symptomatic plants, and plant material and sample if detected. Determine how the nursery disposes of culled plant material. Soil under the cull pile should also be tested for the presence of *P. ramorum*.
- **Equipment**. Determine if equipment used at the facility is shared with other facilities or field areas.
- **Fungicides.** Determine if fungicides are used on the plants at the nursery. If fungicides were used, then record the date, material, amount and application rate. Determine if any other type of treatments (soil amendments, fertilizers) are applied to the plants, soil or growth media.
- **Perimeter survey.** Conduct (initiate and complete) during the nursery site quarantine period, a survey concentrated on plants of all host and associated host genera located within 100-meters of the infested nursery for symptoms of disease caused by *P. ramorum*.
    - Sample all *P. ramorum* symptomatic plants. Samples must be labeled and sent to a laboratory for testing using a method approved by APHIS as stated in Appendix 3.
    - Detection of *P. ramorum* in the perimeter may be indicative of a more widespread infestation. In this case notify your PPQ Regional Office immediately as further regulatory actions may be required.

Disinfest the Nursery

- **Plant Destruction:** Where a *P. ramorum* infected plant(s) is found, all host and associated plants and plant parts within a destruction block will be removed and destroyed using one or more of the techniques detailed in Appendix 1.
- **Non-porous Surfaces:** See Appendix 1 for recommended destruction options.
- **Water Treatment:** If water is contaminated, treatment is imposed (see Appendix 1 for recommended destruction options). The 90 day quarantine period re-starts when water

treatment is initiated. Potting media and water must be retested within the new 90 day quarantine period.

- **Soil and Potting Media Treatment**: See Appendix 1 for recommended destruction options. Soil and or potting media should be sampled within the destruction block and, if either are found infested, treat if necessary. This is the most likely area of soil or potting media infestation (underneath and around the diseased plants, and in containized stock) and the most likely area where reinfestation of new host material would occur at a future date.
- **Equipment and Personnel:** See Appendix 1 for recommended destruction options.
- **Best Management Practices:** See Appendix 2 for recommended management practices designed to control or eliminate the diseases caused by *P. ramorum*.

Release the Nursery

If water and soil and media and perimeter sampling is negative for *P. ramorum*, a nursery may avoid a quarantine period, through a management decision, by voluntarily destroying all hosts and associated host plants and plant parts in the destruction block and the buffer zone.

Nurseries and their plants that have been placed under regulatory control may be released from regulatory control by USDA, APHIS or designated authority after the nursery site quarantine period:

- There are no additional detections of *P. ramorum* in nursery stock based on USDA APHIS approved plant inspection, sampling and testing protocols.
- Water, soil, and potting media have also tested negative for *P. ramorum* based on USDA APHIS approved sampling and testing protocols.

Post eradication monitoring.

Nurseries that have been infested will continue to be monitored for the following two spring seasons as part of the national survey. These nurseries are not under any quarantine or regulatory action, unless additional outbreaks are detected.

Appendix 1

Treatments and Disinfections

The following techniques are approved by USDA APHIS PPQ for control of P. ramorum in nurseries found to contain plants infected with P. ramorum:

Infected Plants – (*Note*: host material, including leaf litter, must not be placed in compost piles or be removed from the facility as trash or in debris removal. Host material should be collected and incinerated or double bagged and deep buried in a site approved by USDA, APHIS or delegated regulatory authority.)

- **Incineration (burning to ash):** Infected plants, associated growth media, associated containers (i.e. pots and trays), all leaf debris in and around the area where plants were stored may be disposed of by incineration at a facility or other location (e.g. on site) approved by USDA and permitted within state and municipal statutes or regulations. Off nursery movement must be properly safeguarded and every effort to prevent plant debris or soil from being dislodged from the plants prior to incineration should be taken. Burning may be through open burning or in an incinerator.

- **Deep burial:** Infected plants, associated growth media, associated containers (i.e. pots and trays), all leaf debris in and around the area where plants were stored must be double bagged using plastic bags of 2 mil thickness or greater and buried to a depth of no less than two meters. The material must be buried at a USDA approved site, onsite, or municipal landfill, which is expected to remain undisturbed. Every effort to prevent plant debris or soil from being dislodged from the plants should be taken.

- **Steam sterilization:** Dry heat or steam commonly heated to internal temperatures of 212^{o} F (100^{o} C) for 30 minutes followed by burial in a landfill, or as otherwise detailed in the USDA Treatment Manual for "insect pests and pathogens in garbage", Schedule T415b (http://www.aphis.usda.gov/ppq/manuals/pdf_files/Treatment%20Chapters/05-05-T400-5.pdf).

- **Composting:** Currently **not** approved by regulation and **not** approved for use in the Confirmed Nursery Protocol or in the Federal Regulations. Recent research shows this to be an efficacious treatment. The procedures tested are codified in California Integrated Waste Management Board regulations (Title 7, Division 7, Chapter 3.1 Article 7, Section 17868.3 (b) and (c)). (See http://www.ciwmb.ca.gov/Regulations/Title14/ch31a5.htm#article7)

Non-Porous Surfaces:

Most disinfectants are not labeled for use in soil and are only useful for nonporous materials such as concrete floors, nursery pots, plastic sheeting. A number of disinfectants are registered for use on nonporous surfaces that may effectively reduce populations of *Phytophthora* species. If it is practicable, tools such as knives, pruners, water breakers, water wands and other implements used in the buffer area should only be used in the buffer area. If tools and other implements must be moved from the buffer area, then regular disinfection using an appropriate disinfectant for the control of *P. ramorum* is recommended prior to removal from the buffer zone. The following table modified from http://cpmcnet.columbia.edu/dept/ehs/decon.html examines the effects of different classes of disinfectants on microbial populations. This list is for

explanation and information only. Few disinfectants are specifically labeled for *Phytophthora* species and are shown in **Bold**.

All labels for the disinfectants listed below must be strictly adhered to for maximum efficacy and environmental and worker safety.

Summary of Disinfectant Activities

Disinfectant	Trade names	Comments	Contact time
Alcohols (ethyl and isopropyl) 60-85%	Lysol Spray	Evaporates quickly so that adequate contact time may not be achieved, high concentrations of organic matter diminish effectiveness; flammable.	10-15 minutes
Phenolics (0.4%-5%)	**Pheno-cen**	Phenol penetrates latex gloves; eye/skin irritant; remains active upon contact with organic soil; may leave residue.	10-15 minutes
Quaternary Ammonium (0.5-1.5%)	**Consan Triple Action 20** **Physan 20** Green-Shield 20	Effective for non-porous surface sanitation (floors, walls, benches, pots). Low odor, irritation. Use according to labels.	10-15 minutes
Chlorine (100-1,000 ppm)	**10% Clorox** **10% Bleach**	Inactivated by organic matter; fresh solutions of hypochlorite (Clorox) should be prepared every 8 hours or more frequently if exposed to sunlight; corrosive; irritating to eyes and skin. **Exposure to sunlight further reduces hypochlorite efficacy**. **Keep solution in opaque container.**	10-15 minutes
Peroxides (0.1-2%)	**Zerotol Oxidate TerraClean**	Inactivated by organic matter, no residual activity. Photosensitive. Use according to labels.	Soil treatment

Water:

- **For dust abatement, fire suppression, and equipment cleaning**: Chlorox (sodium hypochlorite) is labeled (EPA Reg. No 5813-50) for treatment of water (~50 ppm available chlorine) for controlling the spread of *Phytophthora lateralis* via water used for dust abatement, fire suppression and equipment cleaning. The active ingredient level must be measured at the sprinkler head; otherwise the treatment is not effective.
- **For irrigation:** Chlorine levels of 2ppm or 2mg/liter or greater has been correlated with the control of *Phytophthora* spp. in re-circulated irrigation systems. For irrigation purposes, recirculated, non-municipal water, must be chlorinated at an active chlorine concentration

equal to or greater than 2 mg/liter of water; for facilities that recycle water, this chlorine level must be monitored.

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Soil and Potting Media:

- **Potting media**: Potting media must be heated such that the temperature in the center of the load reaches at least 180 degrees F for 30 minutes. Treatment must be conducted in the presence of an inspector or treated with an approved fumigant as detailed below.
- **Soil:** Soil must be heated such that the temperature in the center of the load reaches at least 180 degrees F for 30 minutes. Treatment must be conducted in the presence of an inspector or treated with an approved fumigant as detailed below. Methyl bromide has been used for fumigating wood products, but the data on fungi and related organisms in wood are limited. However, methyl bromide has a long history of fumigation of soil in the field and greenhouse. It has commonly been used in combination with chloropicrin for control of *Phytophthora* spp. and other pests in strawberry beds. Methyl bromide has been used for soil treatment for the mitigation of *P. cinnamoni* in citrus groves. However, many of the compounds currently in use have been implicated in human and environmental risks.

All fumigants are restricted use and must be applied according to labels by a licensed applicator. Any use of restricted pesticides in any manner not listed on the label is unlawful.

Summary of Labeled Soil Fumigants

Fumigant	Trade names	Comments
Chloropicrin	Chlor-O-Pic Metapicrin Timberfume Tri-Clor	Chlorinated hydrocarbon used as a tear gas. Often used in combination with methyl bromide due to its ability to be detected in small quantities. In use as soils fumigant for more that 75 years.
Dazomet	Basamid	Methyl isothyocyanate (MITC) breaks down into cyanide gas. Granular formulation that is water activated.
Metam-sodium	Busan 1020 Busan 1180 Busan 1236 Basamid Vapam	Township caps in California make judicious use of this product a necessity. Limitations in California also include proximity to public places. All applicable labels must be observed.
Methyl Bromide	Tri-Con Terr-O-Gas Preplant Soil Fumigant Pic-Brom	Colorless and odorless. Usually combined in various concentrations with Chloropicrin (tear gas). Use is restricted due to ozone depletion potential. Use to be discontinued in 2005 except for quarantine exemption in accordance with the Montreal Protocol of 1989. Current production of methyl bromide limited to 25% of 1991 levels.

Equipment and Personnel (Inspectors and employees)

- Access to infested areas and hold areas should be limited, as much as possible, to officials and employees. Everyone entering and leaving the facility must scrape off loose pieces of soil. Those working with, or in contact with suspected infested material (including plants), must wash hands using soap or disinfectant immediately after completion of task.

- A disinfectant foot bath should be placed and used by personnel entering and exiting the buffer area at the infested facility, where the movement of soil or plant debris on footwear is likely. The foot bath must be filled with fresh disinfectant on a daily basis. Use of disposable shoe covers may be used in lieu of a footbath, if disposed of immediately upon exit from the buffer. The disposable shoe covers must be placed in bags and incinerated or deep-buried.

- The tires (or other parts in contact with the soil) of vehicles must be cleaned of loose soil before leaving the infested facility.

- Do not visit other commercial operations in potentially contaminated work clothing and footwear. Where it is necessary that visitors enter the facility, the facility should ensure that every precaution is taken to prevent the movement of infected plants, contaminated soil or debris with the visitor.

- Wood surfaces suspected of contamination with *P. ramorum* should be treated with copper naphthenate (Erwin and Ribeiro, 1996).

Appendix 2
Best Management Practices for Nurseries

These Best Management Practices (BMP's) are designed to control or eliminate the diseases caused by *Phytophthora ramorum*.

The control of *P. ramorum* spread is based on the establishment of multiple hurdles or barriers to the pathogen with a purpose of minimizing the risk of introduction or survival of the SOD pathogen in a nursery. The BMP's assure the monitoring of the functionality of the process controls for the pathogen.

Each nursery facility is expected to review these and employ some or all of these practices depending upon their physical location and plant products that are handled. Nurseries are encouraged to incorporate these BMP's into their Standard Operating Procedures.

The BMP's have been divided into three categories:

- Exclusion
- Prevention
- Monitoring

The following BMPs should be considered for preventing the establishment or spread of diseases caused by *P. ramorum*:

Exclusion:

- No over story or under story of known *P. ramorum* hosts on nursery grounds unless there is regular monitoring of those hosts.
- Confirm host stock is propagated from materials originating on site or is received from shipping nurseries (in SOD-quarantined or regulated areas) under compliance agreements.
- All incoming host plants (buy-ins, transfers …), regardless of origin, should be visually inspected for symptoms of *P. ramorum* by trained nursery personnel prior to being incorporated into the production facility.

Prevention:

- Effective fungicide program for the control of *Phytophthora* on susceptible host plants (research in progress, results pending.).
- Off load incoming shipments to an area that can be cleaned of the leafy debris. Sweep debris from the receiving pad and the delivery truck; collect debris and bag for disposal.
- Avoid product returns of nursery stock from a receiver in a quarantined area. If unavoidable, contact your State Regulatory Official (if in California your County Agricultural Commissioner) prior to accepting the nursery stock return.

Monitoring:

- Nursery personnel should attend one or more SOD trainings. Training is available through the California Oak Mortality Task Force, USDA Forest Service, California Department of Food and Agriculture, California County Agricultural Commissioners, and other qualified personnel. SOD training may also be available through State Agriculture Departments, and Universities in other States.
- All host buy-ins should be maintained separately from other hosts plants and periodically inspected for symptoms of the disease over the course of a growing season.

- Monitor host and associated plants in surrounding area for symptoms of *P. ramorum* in Spring and Fall
- Identify sources of disease recognition fact sheets, and/or develop and distribute disease recognition fact sheets on host plants to educate all field nursery personnel.
- Record Keeping: Maintain accurate shipping documentation identifying product, amount, date and origin or receiver for the purpose of identifying trace backs and trace forwards.

If the disease is found in the environs surrounding a nursery, these BMPs should be followed:

- Install diversion burms to prevent soil and water movement, during storm-related events, from hillsides populated with *P. ramorum* host plants.
- Place containers/pots on a soil barrier, such as 6 inches gravel on plastic or other impermeable weed-block or on raised benches.
- Irrigation water from any source other than well or municipal water supplies should be monitored to confirm that it is free from the pathogen.
- Avoid overhead irrigation of host plants where practical. When using overhead irrigation, irrigate in the morning to allow the foliage to dry before nightfall.

Appendix 3
Diagnostics

Samples must be analyzed using a methodology approved by APHIS. See techniques posted at: http://www.aphis.usda.gov/ppq/ispm/sod/survey.htm.

a. **Recommended prescreening**. ELISA prescreening of plant samples is highly recommended to determine the presence of *Phytophthora* spp. The approved ELISA is the test produced by Agdia, Inc.
    1. **Negative prescreening results**. If all samples from a single nursery are found to be negative through ELISA prescreening, no further testing is required. The nursery may be considered free of evidence of *Phytophthora ramorum*.
    2. **Positive prescreening results**. If ELISA prescreening reveals the presence of *Phytophthora* in any plants, each sample that returns positive ELISA results must be tested according to paragraph (b) of this section.

Testing procedures. If ELISA prescreening is not performed, or if results of ELISA prescreening are positive for *Phytophthora* in any sample, the sample must be analyzed using an APHIS-approved nested polymerase chain reaction (PCR) or culture test. Samples are considered positive for *Phytophthora ramorum* based on positive results of a nested PCR test. Positive nested PCR tests do not require confirmatory culture tests, nor do positive culture tests require confirmatory nested PCR tests. Note, however, that if culture tests do not return positive results, a nested PCR test must be conducted, as described below. No culture test is required if a nested PCR test returns negative results.

1. **Nested PCR test**.
    i. **Negative results**. If the results of nested PCR tests are reported negative by APHIS for all samples from a nursery or single shipment, no further testing is required. The nursery or shipment sampled may be considered free of evidence of *Phytophthora ramorum*.
    ii. **Positive results**. If any samples tested using the nested PCR protocol are reported by APHIS as positive results for *Phytophthora ramorum*, the nursery is considered infested.
2. **Culture Test**.
    i. **Negative results**. If the results of culture tests are not positive for *P. ramorum* for any samples taken from a nursery or a single shipment, each plant sample that returns such culture results must be tested again using a nested PCR test, and plants from the nursery or shipment are only eligible for interstate movement in accordance with paragraph (b)(1) of this section.
    ii. **Positive results**. If any culture tests return positive results for *Phytophthora ramorum*, and those results verified by APHIS, the nursery from which they originate is subject to the procedures in this protocol.

Appendix 4
Plant Sampling Protocol

All symptomatic plants must be sampled, collecting and testing a minimum of 40 plant samples per nursery. Samples must be taken from symptomatic plants unless no symptomatic plants are present; if less than 40 symptomatic plants are present, up to 40 asymptomatic plants (or 100% of plants, whichever is less) must be sampled. One sample may contain more than one leaf, but no more than one sample may be taken from a single plant. Samples should be taken from host and associated plants and nearby plants. (or as detailed in 7 CFR 301.92-11(b)(2).)

Appendix 5
Soil and Potting Medium Sampling Protocol
(see http://www.aphis.usda.gov/ppq/ispm/sod/soilsamplingprotocol.html for latest approved protocol)

Soil and Potting Media Sampling

- Infested soil or potting media will look exactly the same as un-infested soil or potting media. Therefore all soil and media must be handled carefully. All tools used to collect soil or media samples must be disinfected with 10% bleach solution, 70% ethanol, or flame-sterilized with a propane torch between blocks. All soil and organic material should be removed from the tools prior to disinfection. Care should also be taken not to transfer soil or potting media from one block to the next on shoes or clothing. All sampling equipment should be cleaned and disinfected prior to entering a new nursery block. Care must be taken to ensure that un-infested soil or potting media is not contaminated by infested soil or potting media.

Preparing for sampling.

- Soil and potting media samples should be collected as composite samples. A composite sample consists of a mixture of sub-samples. Sub-samples (See Figure 1) are small amounts of soil (or media) removed from the ground (or pot) and added together to form a composite sample. The use of sub-sampling increases the chances of finding *P. ramorum* if it is present. Samples should contain a maximum of 500-ml (volume) of soil and/or potting media (½ of a quart-size Ziploc bag). The number of composite samples collected will depend upon the size of the nursery block being sampled (see Table 1). Composite samples of potting media should be kept separate from soil samples. There should be at least two samples, one for potting media and one for soil, unless all plants and associated potting media were destroyed or the plants are not on soil (e.g. on concrete or asphalt). If the surface of soil is covered with gravel take sub-samples from the soil beneath the gravel. If water permeable weed block is present, either covered with gravel or under gravel, the week block should be removed prior to soil sampling.

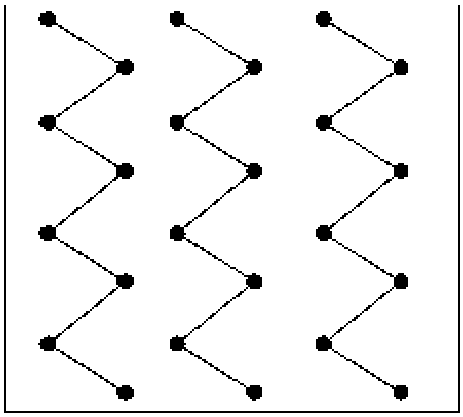
  Table 1. Number of composite samples collected based on nursery block size.

Size of Treated Site (acres)	No. of Soil and Potting Media Samples Collected (total)
0.00 - 0.25	1 (2)
0.26 - 0.5	2 (4)
0.50 - 1.0	4 (8)
1.01 - 2.50	8(16)
>2.51	12 (24)

- Each composite sample will consist of at least five sub-samples collected from soil or potting media within the targeted area. While five is a minimum, it is preferable to take 24 sub-samples of soil or potting media for each sample, provided the area is large enough (for soil samples) and enough plants are present (for potting media samples). Sub-samples should be collected according the pattern in the diagram below (Figure 1). Alternatively, if fallen leaves or other debris from the infected plants are present; sub-sampling may be targeted towards those areas. The location of each composite sample should be maintained (preferably by GPS but at least by flagging) in case follow-up treatment of the soil or potting media for *P. ramorum* is required. Composite samples

may also be collected from neighboring blocks of un-infested plants using the same steps. If you are collecting from blocks of un-infested plants, collect the composite soil/potting media samples from these blocks first to minimize the risk of contaminating un-infested soil/potting media. If all potentially-infested potting media has been destroyed with the infected plants, collect composite samples from the remaining host plants within 2- to 10-m of the originally infected plants that have been placed on hold. Preferentially target the potting media of those plants that are "downstream" (e.g., based on watering patterns) of the originally infected plants.

Figure 1. Recommended pattern for collection of sub-samples for composite soil and/or potting media samples.



Appendix 6
Water Sampling Protocol for Retention Ponds

(see http://www.aphis.usda.gov/ppq/ispm/sod/watersamplingprotocol.html for latest approved protocol)

Phytophthora ramorum is an oomycete, belonging to the group that includes *Pythium* species. Collectively these organisms are called "water molds" and are taxonomically related closer to algae than to fungi. For this reason, water collected from potentially infested nursery blocks must be tested for the presence of *P. ramorum*.

There are two potential methods provided here to detect *Phytophthora* species in water. The first uses rhododendron leaf baits in mesh bags followed by moist chamber incubation of the leaf baits. Any suspect lesions that develop on the rhododendron leaves would be plated on PARP at 18-20°C (64-68°F). Any *Phytophthora* species growing on the PARP would need to be transferred to Corn meal agar or V8 agar for identification to species.

The second method uses water filtration. Water is removed from the pond, filtered with sterile filters and the filters placed on PARP. Once the filter is removed from PARP, any resultant *Phytophthora* colonies are transferred to Corn Meal Agar or V8 agar and identified to species.

- ***In situ* Water Sampling with Rhododendron Leaf Baits**

  A control sample using a leaf bait in distilled water should be run simultaneous with the leaf bait sample in the facility water.

  Prepare the rhododendron leaves as bait by cutting the leaves in a herringbone pattern into (but not through) the mid-vein or by trimming off the petiole end of each leaf. Place 3-4 cut leaves into a mesh bag. Label the bag with a plastic tag listing the date, water source (location), and nursery (i.e., nursery license number). Place the mesh bag into the water source for a minimum of 48-hours to 1-week (preferable). Do not leave the bait in the water source for longer than 1-week as the bait will begin to decompose. Place the bags such that the leaves will remain submerged the entire time (i.e., even if water levels fluctuate within the water source). If possible, place the bait near the influent coming from the area closest to or containing the infested plants.

  Remove the bait from the water source and transfer to a sealable bag for transport to the laboratory. Label the bag with the information on the plastic tag, including the date collected. Log the leaf samples into the appropriate database. Assign a unique sample number to the bait(s) from each nursery.

- **Water Sampling for Filtration**

  Water samples should be collected in a sterile wide-mouth bottle and kept at 5 – 10 C. Water samples should be taken from the surface to increase the likelihood of obtaining zoospores of Phytophthora.

  Sample size should be approximately 1000 ml. Sample should be processed within 48 hours of collection. Number of samples is determined by the size of the nursery pond to be sampled (Table 1)

Table 1. Number of composite samples collected based on pond size.

Size of pond (acres)	No. of water samples collected (liters)
0.00 - 0.25	1
0.26 - 0.5	2
0.50 - 1.0	4
1.01 - 2.50	8
>2.51	12

Appendix 7
Resource and Contact List

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Appendix 8

APHIS List of Hosts and Plants Associated with *Phytophthora ramorum*

(Revision dated 02 August 2004) This list is constantly being updated.
The most current version is posted at: http://www.aphis.usda.gov/ppq/ispm/sod

Proven Hosts for *Phytophthora ramorum*

(These may be regulated in whole or in part – see "Updates" at:
http://www.aphis.usda.gov/ppq/ispm/sod)

Scientific Name (28)	**Common Name**
Acer macrophyllum	Bigleaf maple
Aesculus californica	California buckeye
Arbutus menziesii	Madrone
Arctostaphylos manzanita	Manzanita
Camellia spp.	Camellia - all species, hybrids and cultivars
Hamamelis virginiana	Witch hazel
Heteromeles arbutifolia	Toyon
Lithocarpus densiflorus	Tanoak
Lonicera hispidula	California honeysuckle
Pieris formosa	Himalaya Pieris
Pieris formosa x japonica	Pieris 'Forest Flame', forest flame andromeda
Pieris floribunda x japonica	Pieris 'Brouwer's Beauty', Brouwer's beauty andromeda
Pieris japonica	Japanese Pieris
Pseudotsuga menziesii var. *menziesii*	Douglas-fir
Quercus agrifolia	Coast live oak
Quercus chrysolepis	Canyon live oak
Quercus kelloggii	California black oak
Quercus parvula v. *shrevei*	Shreve's oak
Rhamnus californica	California coffeeberry
Rhododendron spp.	Rhododendron (including azalea) – includes all species, hybrids and cultivars
Rosa gymnocarpa	Wood rose
Sequoia sempervirens	Coast redwood
Trientalis latifolia	Western starflower
Umbellularia californica	California bay laurel, pepperwood, Oregon myrtle
Vaccinium ovatum	Evergreen huckleberry
Viburnum x bodnantense	Bodnant Viburnum
Viburnum plicatum var. *tomentosum*	Doublefile Viburnum
Viburnum tinus	Laurustinus

Plants Associated with *Phytophthora ramorum*

(Until proven as a host by Koch's postulates, there are only regulated as nursery stock and not in other forms. See 7 CFR 301.92 http://www.aphis.usda.gov/ppq/ispm/sod)

Scientific Name (36)	**Common Name, Date & Source of Report**
Abies grandis	Grand fir – June 03 (1)
Aesculus hippocastanum	Horse-chestnut – Dec 03 (3)
Arbutus unedo	Strawberry tree – Dec 02 (7)
Calluna vulgaris	Heath – June 04 (11)
Clintonia andrewsiana	Andrew's clintonia bead lily – May 04 (5)
Castanea sativa	Sweet chestnut – Feb 04 (3)
Corylus cornuta	California hazelnut – Dec 02 (5)
Drimys winteri	Winter's bark – July 04 (3)
Dryopteris arguta	California wood fern – May 04 (5)
Fagus sylvatica	European beech – Dec 03 (3)
Kalmia latifolia	Mountain laurel – Fall 02 (3)
Laurus nobilis	Bay laurel – July 04 (3)
Leucothoe fontanesiana	Drooping leucothoe - Oct 03 (3)
Pieris formosa var. forrestii	Chinese Pieris – Oct 03 (3)
Pieris formosa var. forrestii x Pieris japonica	Pieris – Oct 03 (3)
Pittosporum undulatum	Victorian box – Dec 02 (6)
Pyracantha koidzumii	Formosa firethorn – Apr 04 (9)
Quercus cerris	European turkey oak - Feb 04 (3)
Quercus falcata	Southern red oak – Nov 03 (3)
Quercus ilex	Holm oak – Dec 03 (3)
Quercus rubra	Northern red oak – Nov 03 (8)
Rhamnus purshiana	Cascara – Dec 02 (4)
Rubus spectabilis	Salmonberry – Dec 02 (4)
Salix caprea	Goat willow – July 04 (3)
Smilacina racemosa	False Solomon's seal – June 04 (10)
Syringa vulgaris	Lilac – 2003 (3) updated Oct 03
Taxus baccata	European yew – Aug 03 (3)
Taxus brevifolia	Pacific yew – May 03 (5)
Toxicodendron diversiloba	Poison oak – Dec 02 (4)
Viburnum davidii	David Viburnum - Oct 03 (3)
Viburnum farreri (=V. fragrans)	Fragrant Viburnum – Oct 03 (3)
Viburnum lantana	Wayfaringtree Viburnum – Oct 03 (3)
Viburnum opulus	European cranberrybush Viburnum – Oct 03(3)

Viburnum x burkwoodii	Burkwood Viburnum – Oct 03 (3)
Viburnum x carlcephalum x V. utile	Viburnum – Oct 03 (3)
Viburnum x pragense	Prague Viburnum – Oct 03 (3)

[1] California Department of Food and Agriculture
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Rationale for Lists:

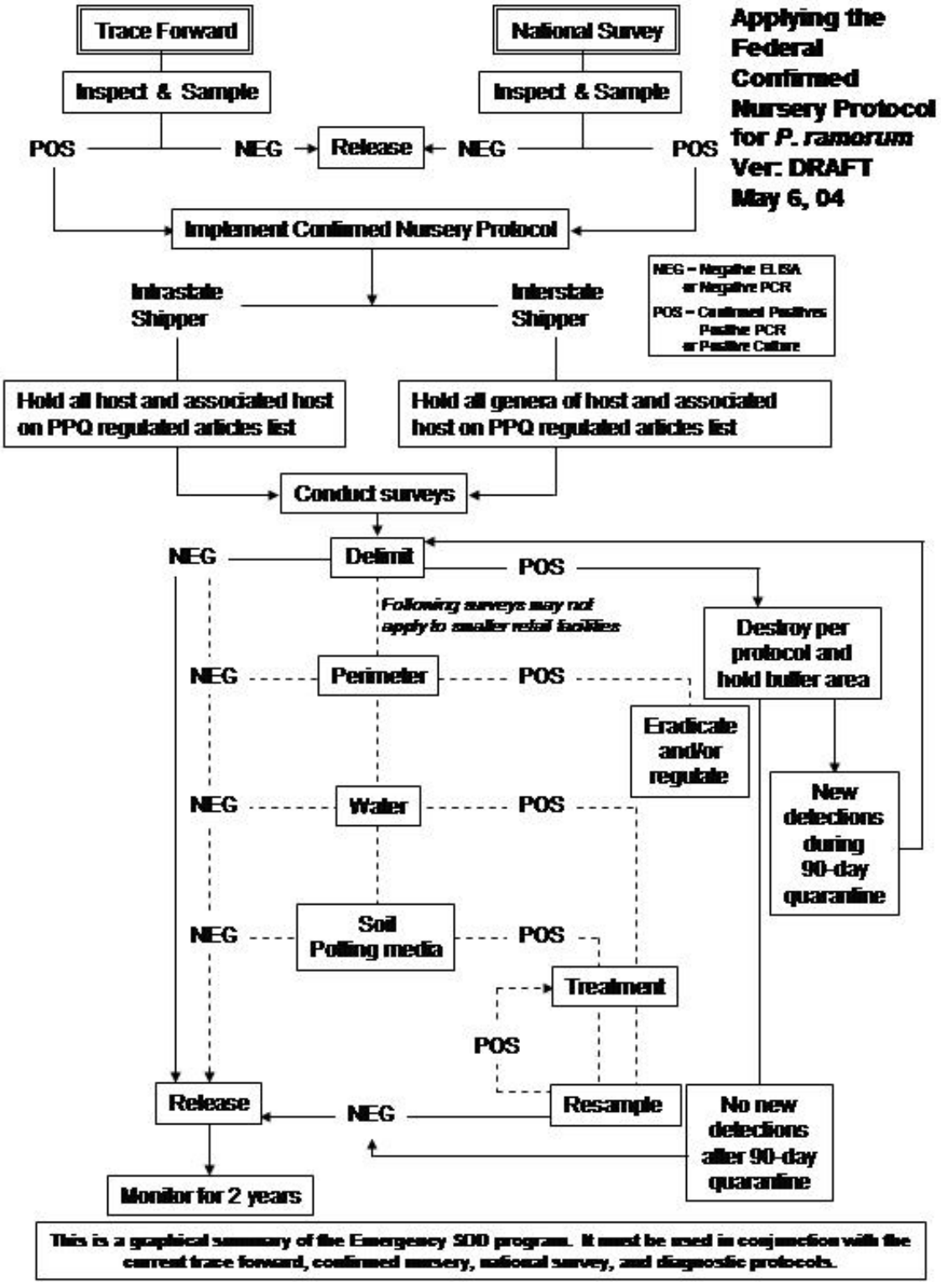
Host Plants for *Phytophthora ramorum:*
Host plants are naturally infected associated plants added upon completion, documentation, review and acceptance of traditional Koch's postulates. Some are regulated in part (such as redwood and Douglas fir); others are regulated in their entirety (such as tanoak and western star flower). Details on regulated plants and articles can be found via links to "Phytophthora ramorum 7 CFR 301.92" and "Recent Modifications to Phytophthora ramorum Regulations" at: http://www.aphis.usda.gov/ppq/ispm/sod

The plants listed in the original Interim Rule dated 14 February 2002 were adapted from a review and evaluation of lists of regulated plants from other regulatory agencies.

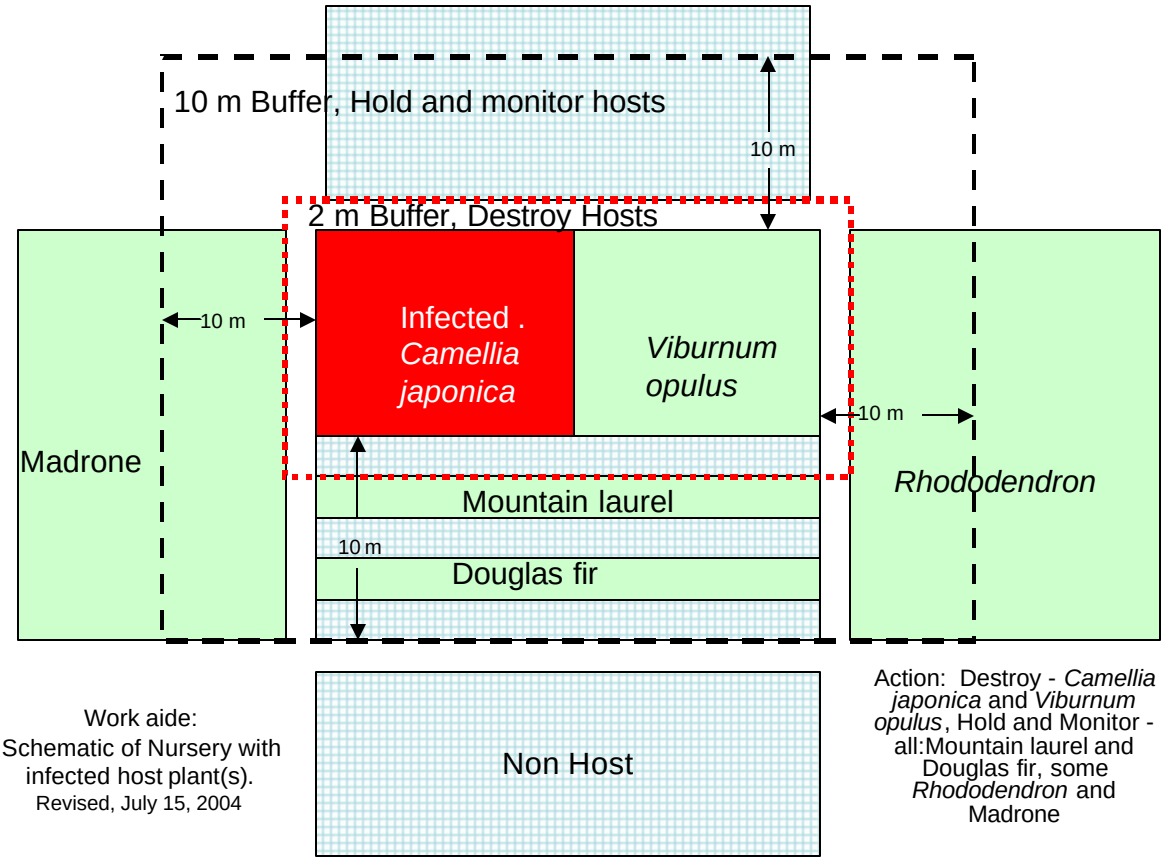
Plants Associated with *Phytophthora ramorum*:
Associated plants are those reported found naturally infected and from which *P. ramorum* has been cultured and/or detected using PCR (Polymerase Chain Reaction). For each of these, traditional Koch's postulates have not yet been completed or documented and reviewed. These reports must be documented and reviewed by PPQ before they will be listed.

Regulation at the genus level:
For either list, a listed plant may be revised to regulate at the genus level to ensure appropriate and effective inspection in quarantine areas, regulated nurseries, and regulated articles to mitigate the spread of *P. ramorum*. An example is when the number of individual species, hybrids, or cultivars listed or to be listed is determined to prevent appropriate and effective inspection or regulation.

Appendix 9
Protocol Schematic



Appendix 10
Work Aide – Schematic of Nursery with Infected Host Plant(s).



Work aide:
Schematic of Nursery with infected host plant(s).
Revised, July 15, 2004

Action: Destroy - *Camellia japonica* and *Viburnum opulus*, Hold and Monitor - all:Mountain laurel and Douglas fir, some *Rhododendron* and Madrone

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